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Chlorination of cellulose with *N*-chlorosuccinimide–triphenylphosphine under homogeneous conditions in lithium chloride–*N*,*N*-dimethylacetamide

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ABSTRACT

Microcrystalline cellulose was chlorinated with *N*-chlorosuccinimide–triphenylphosphine under homogeneous conditions in LiCl–*N*,*N*-dimethylacetamide. At the early stage of the reaction only replacement of the 6-hydroxyl groups with chlorine was observed, and 3-hydroxyl groups were replaced at a lower rate with Walden inversion. The effects of reaction conditions on the extent of chlorination were studied in detail. More than two equivalents of chlorination reagents per glucose residue were necessary to attain a high degree of substitution (ds) by chlorine, and the maximum ds attained was 1.86. Chlorinated disaccharides were found in the hydrolyzates of chlorodeoxycelluloses hydrolyzed under mild conditions, and their structures were studied by mass spectrometry.

INTRODUCTION

Chlorination of cellulose has been studied by many researchers, mostly under heterogeneous conditions[1]. After the discovery of solvent systems for cellulose based on aprotic organic solvents[2], the use of these solvent systems for homogeneous chlorination of cellulose was developed. Nakao et al.[3] were the first to use a solvent system, *N*,*N*-dimethylformamide (DMF)–chloral, for the homogeneous chlorination of cellulose with thionyl chloride. They stated that a chlorodeoxycellulose sample having the degree of substitution (ds, 3 for complete hydroxyl substitution) by chlorine of 2.8 was obtained, but their products were likely contained chloral moieties[4]. Ishii et al.[5] obtained chlorodeoxycelluloses with ds up to 1.3 by homogeneous chlorination with methanesulfonyl chloride in DMF–chloral. Krylova et al.[6] obtained a chlorodeoxycellulose sample of ds 1.7 by chlorination in pyridine–chloroform with sulfuryl chloride. This system became homogeneous at the

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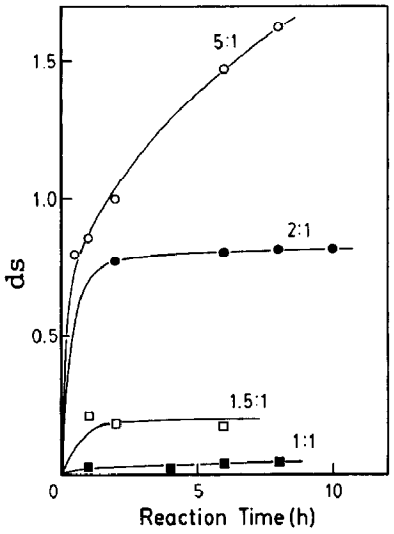


Fig. 1. Effect of reaction time on the extent of chlorination at 50°. Reagent ratios ([NCS, Ph_3P]/[AGU]) are shown in the figure.

later stage of the reaction. Furubeppu et al.[7] attained a ds of 1.8 in the homogeneous chlorination with sulfuryl chloride in lithium chloride–*N*,*N*-dimethylacetamide (DMA). In chlorodeoxycelluloses having high ds values, chlorine atoms were introduced into both C-6 and C-3 positions of glucose residues (AGU groups), and 6-chloro-6-deoxyglucose (6-Cl-Glc) and 3,6-dichloro-3,6-dideoxyallose (3,6-Cl_2-All) residues were formed. The latter was identified by gas chromatographic retention measurements of saccharides in the hydrolyzates[5] and by NMR spectroscopic analysis of chlorodeoxycelluloses[6].

We reported preliminary results on the homogeneous chlorination of microcrystalline cellulose with *N*-chlorosuccinimide (NCS)–triphenylphosphine (Ph_3P) in LiCl–DMA[8]. The LiCl–DMA solvent system is much easier to handle as compared with other systems such as DMF–chloral[9]. Here we describe this reaction in detail. The effects of reaction conditions on the ds of the product are discussed first, and the mass-spectrometric analysis of component saccharides are described next.

RESULTS AND DISCUSSION

Chlorination.—Equimolar amounts of NCS and Ph_3P were used throughout this study. Fig. 1 shows the effect of reaction time on the extent of chlorination at several levels of reagent ratio ([NCS, Ph_3P]/[AGU]). The ds of the product increased rapidly at the early stage of the reaction, and leveled off after ~ 2 h at a reagent ratio up to 2:1. The level-off ds was a function of the reagent ratio, and became higher as the reagent ratio increased. At the reagent ratio of 5:1, the ds continued to increase significantly, even at the later stages of the reaction, and exceeded 1.5.

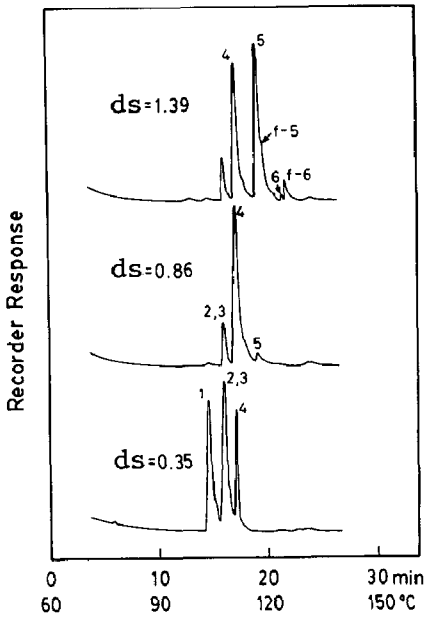


Fig. 2. Gas chromatograms of hydrolyzates of chlorodeoxycelluloses with different ds values. Peaks 1 and 2, Glc*p*; 3 and 4, 6-Cl-Glc*p*; 5 and 6, 3,6-Cl_2-All*p*; f-5 and f-6, 3,6-Cl_2-All*f*.

To determine the chemical structures of the products, chlorinated samples were hydrolyzed in sulfuric acid and the hydrolyzates converted into volatile *O*-trifluoroacetyl (TFA) derivatives in dichloromethane for gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) analyses. TFA derivatives[10–17] are recommended for GC analysis of saccharides because of their high volatility as compared with trimethylsilyl, acetyl, and other derivatives[18].

Anomeric pyranose isomers were found on the chromatograms (Silicone GE SE-30 as the stationary phase) for all of the TFA saccharides studied. For most of the TFA monosaccharides, furanose isomers were also detected. Fig. 2 shows chromatograms of hydrolyzates of chlorodeoxycelluloses having different ds values. The α- and β-anomers of D-glucopyranose correspond to peaks 1 and 2, respectively. Pyranose anomers of 6-Cl-Glc correspond to peaks 3 (overlapped with peak 2) and 4. Peaks 5 and 6 are due to pyranose anomers of 3,6-Cl_2-All, and peaks f-5 and f-6 are attributable to furanose anomers. This saccharide was identified by comparing its GC retention values and mass spectrum with those of an authentic sample[5,19]. At the early stages of reaction, only replacement the 6-hydroxyl groups of the glucose residues with chlorine was observed. When the ds of the chlorodeoxycellulose exceeded ~ 0.8, 3,6-Cl_2-All began to appear on the chromatograms. This indicate that substitution of the 3-hydroxyl groups occurred, with Walden inversion, at a much lower rate than that of C-6 hydroxyl groups.

Fig. 3 shows the effect of reaction temperature on the extent of chlorination under two different reaction conditions. At a low reagent ratio (2:1), only the C-6

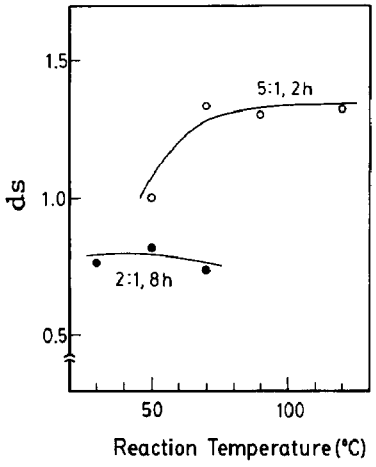


Fig. 3. Effect of reaction temperature on ds. Reaction conditions are shown in the figure.

hydroxyl groups were replaced by chlorine. After a sufficiently long reaction-time (8 h), the reaction temperature showed little effect on the ds. In contrast, at a reagent ratio of 5:1 and in a relatively short reaction time (2 h), the ds was lower at 50° than those observed at higher temperatures. This is attributable to the slow replacement of C-3 hydroxyl groups with chlorine at this reagent ratio. The recovery of the chlorination product decreased at 90° or higher and was < 50% at 120°. The products obtained at higher temperatures were extensively colored.

Fig. 4 shows the effect of the reagent ratio on the extent of chlorination for 6 h at 50°. The ds increased steeply when the reagent ratio was increased from 1:1 to 3:1. The maximum ds in this case was 1.63. When the concentration of lithium chloride was increased from 1.18 to 1.77 mol/L, the ds further increased; a ds as high as 1.86 was obtained in the reaction for 2 h at 70° at a reagent ratio of 5:1.

Fig. 5 shows infrared spectra of the original cellulose and chlorodeoxycelluloses with different ds values. For the chlorinated samples, two new absorptions ap-

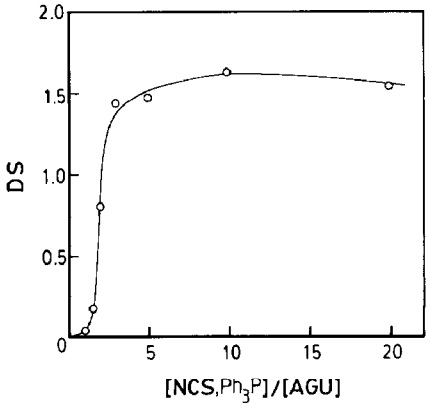


Fig. 4. Effect of reagent ratio on ds. Reaction conditions: 50°, 6 h, [LiCl] 1.18 mol/L.

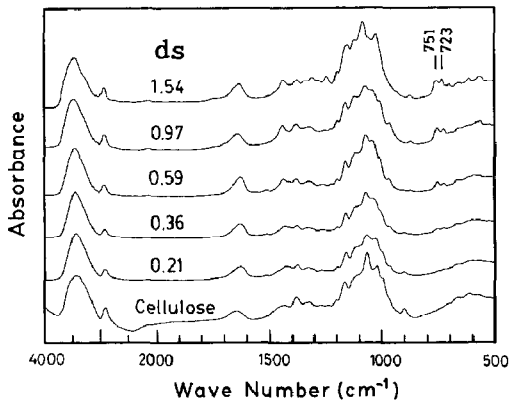


Fig. 5. Infrared spectra of cellulose and chlorodeoxycelluloses with different ds values.

peared in the $\nu_{C\text{-}Cl}$ region[20], 751 and 723 cm^{-1}, and their intensities increased with increase in ds. Fig. 6 shows the relationship between ds and the absorbances of two absorptions, which were normalized with that of the $\nu_{C\text{-}H}$ absorption at 2923 cm^{-1}. The intensities of both absorptions increased linearly with the increase in ds up to ~ 1. This finding shows that these absorptions are due to $\nu_{C\text{-}Cl}$ vibrations, as suggested by Shimizu et al.[4]. In the high-ds region, where 3,6-Cl_2-All appeared on the chromatograms of the hydrolyzates, the absorbance of the 751-cm^{-1} band tended to level off while that of the 723-cm^{-1} band increased at a lower rate with the increase in ds. The C-3 chlorine atoms in 3,6-Cl_2-All units are axial, and axial $\nu_{C\text{-}Cl}$ absorptions appear at lower wavenumbers[20]. One of the possible explana-

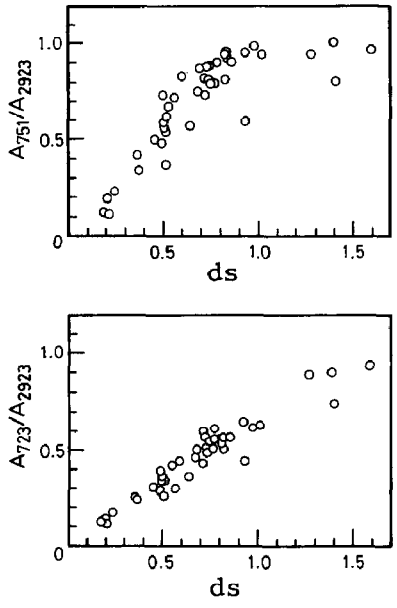


Fig. 6. Absorbance ratios for absorptions at 751 and 723 cm^{-1} as a function of ds.

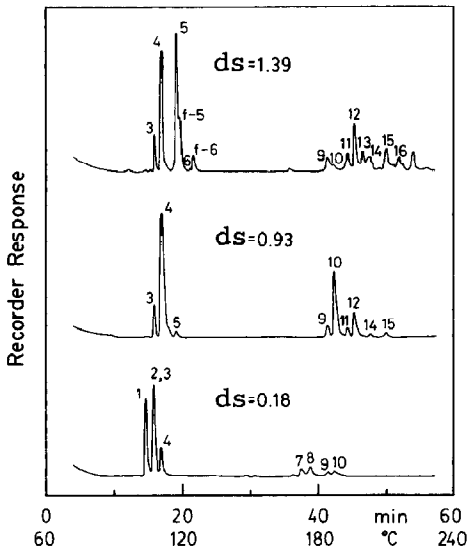
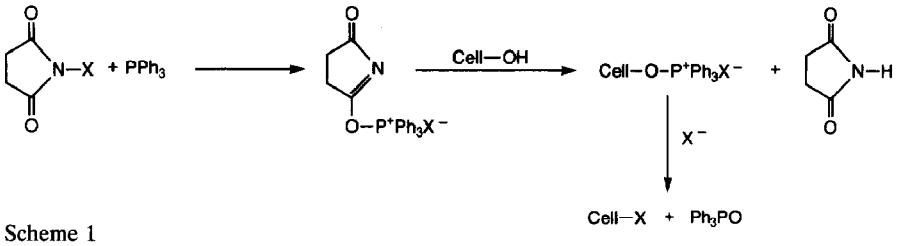


Fig. 7. TIC chromatograms of hydrolyzates of chlorodeoxycelluloses with different ds values hydrolyzed under mild conditions. Peaks 1 and 2, Glc*p*; 3 and 4, 6-Cl-Glc*p*; 5 and 6, 3,6-Cl_2-All*p*; f-5 and f-6, 3,6-Cl_2-All*f*; 7 and 8, 6′-Cl-cellobiose; 9 and 10, 6,6′-Cl_2-cellobiose; 11 and 12, Cl_2-Cl_1; 13 and 14, Cl_1-Cl_2; 15 and 16, Cl_2-Cl_2. Hydrolysis conditions, see text.

tions for the behavior of $\nu_{C\text{-}Cl}$ absorptions is that they appear at $\sim 20\ cm^{-1}$ lower wavenumbers with decreased extinction coefficients when they are axial.

Fig. 7 shows the chromatograms of the hydrolyzates of chlorodeoxycelluloses of different ds values hydrolyzed under mild conditions. Disaccharide peaks appeared at higher column temperatures. For the sample having ds 0.18, anomers of 4-*O*-(6-chloro-6-deoxy-β-D-glucopyranosyl)-D-glucose (6′-Cl-cellobiose, peaks 7 and 8) and 4-*O*-(6-chloro-6-deoxy-β-D-glucopyranosyl)-6-chloro-6-deoxy-D-glucose (6,6′-Cl_2-cellobiose, peaks 9 and 10) appeared on the chromatogram, along with glucose and 6-Cl-Glc. 6,6′-Cl_2-cellobiose was identified by comparing its GC retention values and mass spectrum with those of an authentic sample[21].

The peak areas of chlorodeoxycellobioses were relatively large, despite the low ds of the sample. For the sample having ds 0.93, peaks due to tri- and tetra-chlorinated disaccharides (peaks 11–15) appeared on the chromatogram. Although the total area of the 6,6′-Cl_2-cellobiose peaks was the largest among those of disaccharides, the peak areas of tri- and tetra-chlorinated disaccharides were larger than expected for the ds of the sample. In fact, only a small amount of 3,6-Cl_2-All was found on the chromatograms of the hydrolyzate of the sample after complete hydrolysis. In the present study, 6′-Cl-cellobiose was found, but 4-*O*-β-D-glucopyranosyl-6-chloro-6-deoxy-D-glucose could not be detected. These findings may be explained in terms of the increased resistance of the glycosidic bonds in chlorinated saccharides towards acid hydrolysis[22].



Scheme 1

The *N*-halosuccinimide–Ph_3P system, especially *N*-bromosuccinimide–Ph_3P, was successfully used for the halogenation of primary hydroxyl groups in saccharides[23]. It also halogenates secondary hydroxyl groups under suitable conditions with Walden inversion[24]. The halogenation of cellulose by this reagent system is considered to proceed through the formation of cellulose phosphonium salts followed by the attack of halide ions on the phosphonium ester bonds (Scheme 1)[1,24].

Both the formation of the phosphonium ion and the nucleophilic substitution by the halide ion will be affected by the reaction conditions. The tendency for the formation of phosphonium esters of the three hydroxyl groups in glucose residues is considered to be closely related to the tendencies for formation of other esters or ethers. The general order of the ease of formation of esters or ethers is considered[25] to be C-6 $\geqq$ C-2 $\gg$ C-3. The reactivities of modified hydroxyl groups towards nucleophilic substitutions with anions decrease generally in the following order[26], C-6 > C-3 $\gg$ C-2 $\approx$ 0. The experimental findings concerning the effects of reaction conditions on the extent of chlorination are consistent with the differences in reactivity among hydroxyl groups towards ester formation and subsequent nucleophilic substitution. At reagent ratios < 2:1 (Fig. 4), the extent of chlorination was low and only a small amount of 6-Cl-Glc was detected in the hydrolyzates. The ds increased to 0.81 at 2:1 and most of the glucose residues were converted into 6-Cl-Glc units. At this reagent ratio, however, the dichlorination of glucose residues to 3,6-Cl_2-All units was not observed. At the reagent ratio of 3:1, 3,6-Cl_2-All units were formed to a considerable extent. At higher reagent-ratios and at higher temperatures, the substitution of 3-hydroxyl groups by chloride ions proceeded further and the ds exceeded 1.5. The very low reactivity of the C-2 hydroxyl groups towards nucleophilic substitutions with anions may be explained in terms of steric and electronic effects[26]. In the literature, 3,6-Cl_2-All has been identified as a dichlorinated monosaccharide in the chlorination of cellulose, whereas the corresponding substitution product at C-2, 2,6-dichloro-2,6-dideoxymannose, has not yet been found. In the present study also, only the former saccharide was found in the hydrolyzates of the products having high ds values.

Mass-spectrometric analysis.—Mass spectra of TFA derivatives of mono- and di-saccharides were first reported by König et al.[16]. Table I summarizes the MS

TABLE I

Mass spectra of some saccharides as the *O*-trifluoroacetyl derivatives [a]

Fragment	Peak 1		Peak 4		Peak 5		Peak f-6	
	m/z	r.a.	*m/z*	r.a.	*m/z*	r.a.	*m/z*	r.a.
M^+	660	0.0	582	0.0	504	0.0	0504	0.0
A_1	547	0.1	469	0.3	391	3.7	391	5.1
A_2	433	0.1	433	0.1	355	2.5	355	2.2
			355	0.6	277	2.3	277	2.9
A_3	319	13.7	319	2.0	319	0.3	319	0.9
			241	14.3	241	10.6	241	12.8
					163	1.8	163	2.9
E_1	533	0.1	533	0.6	455	3.8	329	18.2 [b]
E_2	419	0.3	419	1.3	419	0.1	293	1.0
					341	1.3	215	26.6
E_3	305	5.5	305	14.3	305	2.4	179	0.6
					227	3.0	101	4.8
C_2	405	0.2	405	1.7	405	0.1	405	0.1
					327	9.2	327	7.0
F_1	265	6.7	265	30.1	265	35.0	265	51.9
					187	5.7	187	28.8
CF_3CO	97	18.8	97	21.5	97	23.5	97	17.1
C_5H_5O	81	10.4	81	10.5	81	14.6	81	13.2
CF_3	69	100	69	100	69	100	69	100

[a] Mass numbers for 6-Cl-Glc and 3,6-Cl_2-All are based on ^{35}Cl. [b] Overlapped with ^{37}Cl-C_2: r.a., relative abundance (%); peak 1, glucopyranose; peak 4, 6-Cl-Glc*p*; peak 5, 3,6-Cl_2-All*p*; peak f-6, 3,6-Cl_2-All*f*. Nomenclature for fragment ions, see text and ref. 27.

fragmentation patterns of TFA derivatives of glucopyranose, a pyranose tautomer of 6-Cl-Glc, and pyranose and furanose tautomers of 3,6-Cl_2-All. Several series of fragmentations due to the loss of trifluoroacetic acid molecules are characteristic of TFA derivatives. The nomenclature proposed by Chizhov and Kochetkov[27] for the various series of fragment ions is used here in a modified form[28]. The structures of most of the fragment ions may be explained based on the fragment ions of corresponding *O*-methyl or *O*-acetyl derivatives[27]. The assignments were confirmed by the isotopic patterns due to chlorine substitutions. Both A and E series are present for the TFA aldohexopyranoses. The former starts with the loss of the anomeric $OCOCF_3$, and the latter starts with the loss of CH_2X (X = $OCOCF_3$ or Cl). A series starting with the loss of $CH(OCOCF_3)$-CH_2X moiety is characteristic of furanose tautomers (E series for furanose), and the series of ions originating from the loss of CH_2X are absent.

Table II summarizes MS fragmentation patterns of TFA disaccharides. The structures of component saccharides could be determined readily, because the series of ions due to reducing and non-reducing ends (bA_1 and aA_1, respectively) and their daughter ions, which were formed by the loss of trifluoroacetic acid and/or hydrogen chloride molecules, appeared clearly in the mass spectra. The ions of the baA and baE series were usually very weak (Scheme 2).

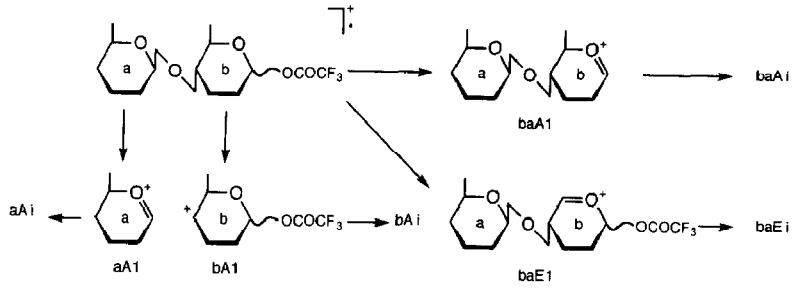
TABLE II

Mass spectra of disaccharides as the *O*-trifluoroacetyl derivatives [a]

Fragment	Cellobiose		Peak 7		Peak 10		Peak 12		Peak 13		Peak 15	
	m/z	r.a.	*m/z*	r.a.	*m/z*	r.a.	*m/z*	r.a.	*m/z*	r.a.	*m/z*	r.a.
M^+	1110	n.m.	1032	n.m.	954	0.0	876	n.m.	876	n.m.	798	0.0
baA_1	997	0.0	919	n.m.	841	0.0	763	0.3	763	0.3	763	3.7
											685	0.2
baA_2	883	0.7	805	n.m.	805	0.0	727	0.6	727	0.2	649	0.4
					727	0.4	649	0.4	649	0.2	571	0.0
baA_3	769	1.5	691	1.4	691	0.1	613	0.5	613	0.0	535	0.4 [b]
					613	0.1	535	0.8	535	0.4 [b]	457	0.0
baA_4	655	0.1	655	0.0	577	0.0	499	0.0	499	0.0	421	0.0
			577	0.0	499	0.0	421	0.0	421	0.2	343	0.0
baE_1	983	0.0	905	n.m.	905	0.0	827	n.m.	827	n.m.	749	0.0
baE_2	869	0.0	791	n.m.	791	0.0	791	n.m.	791	n.m.	713	0.0
							713	0.2	713	0.1	635	0.0
baE_3	755	0.4	677	0.0	677	0.2	677	0.0	677	0.0	599	0.0
							599	0.3	599	0.2	521	0.0
baE_4	641	0.3	641	0.4	641	0.0	563	0.0	563	0.0	485	0.5
			563	0.0	563	0.05	485	1.2	485	0.0	407	0.7
abJ_1	689	0.1	689	0.3	611	0.1	611	0.2	533	0.3	533	0.2
abJ_3	479	0.6	479	2.4	401	1.4	401	10.1	323	1.1	323	6.9
aA_1	547	11.7	469	10.2	469	8.0	391	35.3	469	16.6	391	100
aA_2	433	0.8	433	0.9	433	0.1	355	22.1	433	0.0	355	4.3
			355	2.3	355	15.8	277	10.7	355	7.5	277	30.8
aA_3	319	100	319	99.1 [c]	319	1.5	241	95.5	319	0.8	241	68.8
			241	100	241	100	163	5.4	241	100	163	13.0
bA_1	547	11.7	547	26.5	469	8.0	469	8.6	391	5.0	391	100
bA_2	433	0.8	433	0.9	433	0.1	433	0.0	355	7.5	355	4.3
					355	15.8	355	22.1	277	10.7	277	30.8
bA_3	319	100	319	99.1	319	1.5	319	2.7	241	100	241	68.8
					241	100	241	95.5	163	3.7	163	13.0
bB_2	407	0.8	407	1.6	407	7.1	407	9.2	329	29.2 [d]	329	41.8 [d]
aC_2	405	6.4	405	8.1	405	3.0	327	96.1	405	1.2	327	76.0
			327	16.8	327	14.5	249	2.3	327	12.6	249	5.7
C_5H_5O	81	19.5	81	25.5	81	15.6	81	41.7	81	11.4	81	12.0
CF_3	69	54.8	69	88.4	69	43.8	69	100	69	49.1	69	61.1

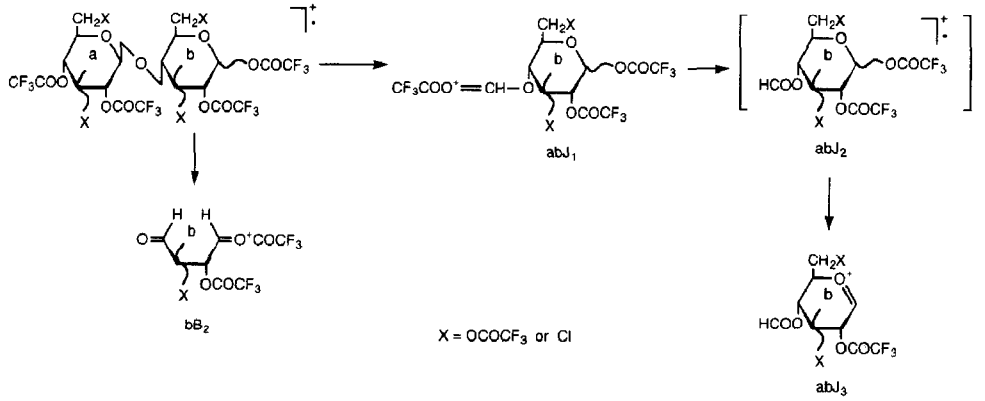
[a] Mass numbers are based on ^{35}Cl: r.a., relative abundance (%); n.m., not measured; peak 7, 6′-Cl-cellobiose; peak 10, 6,6′-Cl_2-cellobiose; peak 12, Cl_2-Cl; peak 13, Cl_1-Cl_2; peak 15, Cl_2-Cl_2. Nomenclature for fragment ions, see text and refs. 27 and 28. [b] Overlapped with ^{37}Cl-abJ_1. [c] Mainly from bA_3. [d] Overlapped with ^{37}Cl-aC_2.

The sequence in oligosaccharides may also be determined by inspection of appropriate fragment-ions[27,28]. In this study, the following ions were used for the structural determination of disaccharides. The ions appearing at *m/z* 689, 479, and 407 were characteristic of TFA disaccharides, composed of aldohexoses, such as TFA-cellobiose and TFA-maltose. These ions were absent in the mass spectra of TFA-aldohexopyranoses. For 6,6′-Cl_2-cellobiose, the ions at *m/z* 689 and 479 were absent and two doublets appeared at *m/z* 611/613 and 401/403 (3:1, corresponding to the replacement, $OCOCF_3 \rightarrow Cl$) along with the ion at *m/z* 407.



Scheme 2

In the mass spectrum of TFA-[3-*O*-β-D-galactopyranosyl-D-arabinose], the ions at *m/z* 689 and 479 were absent and corresponding ions appeared at *m/z* 563 and 353, respectively ($CH_2OCOCF_3 \rightarrow H$). This finding indicates that these two ions were derived from the reducing ends of disaccharides. The ion at *m/z* 689 for TFA-cellobiose and TFA-maltose corresponds to abJ_1 ions (Scheme 3) of *O*-methyl[27,28], *O*-acetyl[28], and *O*-trimethylsilyl[28–30] derivatives. The ion at *m/z* 479 corresponds to an abJ_3 * ion. This ion has not been definitely described for oligosaccharides in the literature. In the mass spectrum of TFA-lactose reported by König et al.[16], the ion at *m/z* 479 was clearly shown in a definite intensity, although they gave no comment on this ion. The ion at *m/z* 317 reported for disaccharide *N*-benzylglycosylamine acetates[29] corresponds to the abJ_3 ion in an *O*-acetyl form, but no explanation was given for this ion. The abJ_2 ions were reported for oligosaccharides of *N*-arylglycosylamine acetates[29]. For TFA derivatives, however, the abJ_2 ion was not observed. The ion at *m/z* 407 for TFA-cellobiose, TFA-maltose, and TFA-[6,6′-Cl_2-cellobiose] corresponds to the bB_2 ion of



Scheme 3

* The term abJ_3 is used in this study to keep consistency of this fragmentation series, although a different term such as baX would be better for this ion.

O-methyl derivatives of (1 → 4)-disaccharides[27,28], which is also formed from the reducing end.

In the mass spectra of peak 7 and 8 (Fig. 7), two series of ions due to TFA-glucose and TFA-6-Cl-Glc moieties were observed with strong intensities. In addition, ions at *m/z* 689, 479, and 407 were observed, but the doublets at *m/z* 611/613 and 401/403 were absent. The peak material was therefore determined to be anomers of 6′-Cl-cellobiose. In the mass spectra for peaks 11 and 12, two ions due to TFA-6-Cl-Glc and TFA-3,6-Cl_2-All moieties were observed with strong intensities along with the ion at *m/z* 407 and the doublets at *m/z* 611/613 and 401/403. The peak materials was determined to be anomers of 4-*O*-(3,6-dichloro-3,6-dideoxy-β-D-allopyranosyl)-6-chloro-6-deoxy-D-glucose (Cl_2-Cl_1). In the mass spectra for peaks 13 and 14, two series of ions due to TFA-6-Cl-Glc and TFA-3,6-Cl_2-All moieties were also observed along with triplets (*m/z* 533/535/537 and 323/325/327) that correspond to the ions at *m/z* 689 and 479, respectively ($2 \cdot OCOCF_3 \rightarrow 2 \cdot Cl$), and a doublet (*m/z* 329/331) corresponding to the ion at *m/z* 407 ($OCOCF_3 \rightarrow Cl$). The peak material was determined to be anomers of 4-*O*-(6-chloro-6-deoxy-β-D-glucopyranosyl)-3,6-dichloro-3,6-dideoxy-β-D-allose (Cl_1-Cl_2). In the mass spectra for peaks 15 and 16, only the series of ions due to the TFA-3,6-Cl_2-All moiety appeared, along with the triplets at *m/z* 533/535/537 and 323/325/327 and the doublet at *m/z* 329/331. The peak material was determined to be anomers of 4-*O*-(3,6-dichloro-3,6-dideoxy-β-D-allopyranosyl)-3,6-dichloro-3,6-dideoxy-D-allose (Cl_2-Cl_2).

Guerrera and Weill[31] studied the mass spectra of maltose derivatives, mainly as *O*-acetyl derivatives, and concluded that the intensity of the oxonium ion derived from the non-reducing end (aA_1) was always stronger than that of the carbonium ion derived from the reducing end (bA_1) because of the higher stability of the former. Their conclusion, however, is contradictory in some cases to the present way of assignment which is based on the abJ_1, abJ_3, and bB_2 ions. In the mass spectra of disaccharides whose structures were assigned according to the present method, the intensity of the aA_1 ion was not always stronger than that of the bA_1 ion, although the intensities of both ions were much higher than those of corresponding A_1 ions observed in the mass spectra of TFA monosaccharides. This is probably due to the subsequent elimination of trifluoroacetic acid and/or hydrogen chloride molecules from these ions to form daughter ions with benzenoid structures. In this case, the stability of the benzenoid cation is important, and the relative intensity of the mother ion will be decreased if the formation of the daughter ions is facilitated.

Krylova et al.[6] concluded, based on the NMR spectroscopic analysis of chlorodeoxycelluloses, that the main repeating unit in the chlorodeoxycelluloses having ds values about 1 was the 6-Cl-Glc unit. In the samples with higher ds values, on the other hand, the linkages between 6-Cl-Glc and 3,6-Cl_2-All units were predominant. In the present study, several disaccharides with high ds values were found in the hydrolyzates of chlorodeoxycelluloses. Although most of the

assigned structures have not yet been referred to authentic samples for definite assignment, the relative amounts of these will offer, after unequivocal determination of their structures, useful information such as the distribution of chlorine atoms along the cellulose chains and the hydrolytic reactivity of glycosidic bonds between hexose units with various levels of chlorination.

EXPERIMENTAL

Reagents.—Microcrystalline cellulose (Art. 2331 Cellulose mikrokristallin, Merck) was dried in a desiccator under diminished pressure before use. *N*-chlorosuccinimide and Ph_3P were recrystallized from water and EtOH, respectively. *N*,*N*-Dimethylacetamide (DMA) was dehydrated with calcium hydride, distilled under diminished pressure and stored over molecular sieves (Linde Type 4A). Lithium chloride was dried over silica gel under diminished pressure at room temperature. Commercial D-glucose (α and β), 6-Cl-Glc, cellobiose, maltose, and 3-*O*-β-D-galactopyranosyl-D-arabinose were used without further purification. Methyl 3,6-dichloro-3,6-dideoxy-β-D-allopyranoside was synthesized according to the method of Dean et al.[19], and hydrolyzed[5] to 3,6-Cl_2-All in 8% H_2SO_4 under reflux for 2 h. 6,6′-Dichloro-6,6′-dideoxycellobiose was synthesized according to the method of Thiem[21].

Chlorination.—The dissolution and chlorination of cellulose were carried out under N_2. Microcrystalline cellulose (0.1 g) was added to a flask containing 10 mL of DMA, and the mixture was heated for 1 h at 165° with stirring. The temperature was lowered to 90° and 1.0 g (23.6 mmol) of LiCl was added. After stirring for 1 h, the temperature was lowered to 60° and the mixture was stirred further at this temperature. A clear homogeneous solution was obtained within several hours.

In a typical chlorination, calculated amounts of NCS and Ph_3P (both in DMA solutions, [NCS]:[Ph_3P] = 1:1) were added in this order to the solution mentioned above under cooling with ice–water. The final volume of DMA was 20 mL. The solution was then stirred at room temperature for about 15 min and held at the reaction temperature for the required time with stirring. After chlorination, the solution was poured into 400 mL of acetone, and the precepitated material was washed with acetone, dialyzed against distilled water for 2 days, washed with MeOH and dried under diminished pressure.

Analyses.—Chlorine contents of the products were determined by an oxygen-flask combustion method[32]. The degree of chlorine substitution was calculated from the chlorine content.

Infrared spectra were recorded in KBr disks on a Fourier-transform IR spectrophotometer FT/IR-3 (Nihon Bunko Co.). A spectrum of ambient air was used for the reference.

For gas chromatography and gas chromatography–mass spectrometry, cellulose samples were hydrolyzed in H_2SO_4. For complete hydrolysis, a sample (20 mg) was immersed in 1 mL of 72% H_2SO_4 at 5° for > 48 h until dissolution was complete.

For samples with high ds values, a decreased amount of sample and a longer period of immersion were necessary. The solution was diluted to 8% H_2SO_4 and refluxed for 2 h. For partial hydrolysis, three conditions were adopted: (*A*) a sample (ds 0.18) was immersed in 72% H_2SO_4 for 2 h at 6°, diluted to 8%, and refluxed for 1 h; (*B*) a sample (ds 0.93) was immersed in 72% H_2SO_4 for 48 h at 6°, diluted to 8%, and refluxed for 2 h; (*C*) a sample (ds 1.39) was immersed in 72% H_2SO_4 for 48 h at 6°, diluted to 8%, and refluxed for 0.5 h. The hydrolyzate solutions were neutralized with an aq $Ba(OH)_2$ to pH 6–7. The $BaSO_4$ formed was removed by centrifugation, and the supernatant solutions were evaporated to dryness. The saccharides were converted into *O*-TFA derivatives in a Pierce Reacti-Vial by the reaction with trifluoroacetic anhydride in CH_2Cl_2 for 20 min at 100°.

Gas chromatograms were recorded on a Shimadzu GC-4BM dual-column gas chromatograph equipped with two flame-ionization detectors. A glass column (3 m × 3 mm, i.d.), was used, packed with 3% Silicone GE SE-30 on Gas Chrom Q (100–120 mesh). The flow rate of the carrier gas (N_2) was 60 mL/min, and the column temperature was raised from 60 to 250° at 3°/min. A Shimadzu Chromatopac C-E1B was used for the data analysis.

A Shimadzu LKB-9000 gas chromatograph–mass spectrometer was used for the GC–MS analysis. The temperatures of the ion source and the separator were 290 and 280°, respectively. The total-ion-current (TIC) chromatograms were recorded at an acceleration voltage of 20 eV, and the mass spectra were measured at 70 eV. The flow rate of the carrier gas (He) was 30 mL/min, and other conditions for analysis were the same as those for the GC analysis. A Shimadzu GC-MSPAC 300 data processor was used for the data analysis.

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